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Effect of aeration and agitation on volumetric oxygen transfer in *Pichia pastoris* culture system

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The oxygen mass transfer coefficient (K_La) often serves to compare the efficiency of bioreactors and their mixing devices as well as being an important scale up factor. In submerged fermentation, four methods are available to estimate the overall oxygen mass transfer coefficient. Each method provides a distinct estimation of the value of K_La. Dynamic gassing out method was used to obtain a more probable value of K_La during the fermentation in 5L fermentor with *Pichia pastoris*. The aeration and agitation were varied to study the effect on K_La. Results showed that the K_La value increased with increase in agitation and aeration. While working in 5L fermentor with *Pichia pastoris* the maximum K_La value of 441.36 was obtained when the agitator speed 300 RPM and aeration 1.5 vvm was maintained. This shows that the volumetric oxygen transfer rate varies in variation with aeration and agitation and not based on organism used. The optimum conditions of agitation and aeration to achieve maximum K_La for a given microorganism depends on its shear sensitive nature.

Key words : K_La, *Pichia pastoris*, Aeration, Agitation, Dissolved oxygen.

INTRODUCTION

The supply of oxygen is critical factor in all aerobic fermentations. An insufficient oxygen transfer leads to a decrease of microbial growth and product formation. In order to assess if particular equipment would be able to supply oxygen at a non-limiting rate, it is essential to have a good estimate of oxygen mass transfer coefficient (K_La). In submerged fermentation oxygen mass transfer coefficient serves to compare the efficiency of bioreactors and their mixing devices. It is also one of the important scale-up factors.

Hirose and Shibai's (1980) investigations of aminoacid biosynthesis by *Brevibacterium flavum* provide an excellent example of the effect of the dissolved oxygen concentration on the production of a range of closely related metabolites. These workers demonstrated the critical dissolved oxygen concentration for *B. flavum* to be 0.01 mg dm^{-3} and considered the extent of oxygen supply to the culture in terms of the degree of 'oxygen satisfaction', that is the respiratory rate of the culture expressed as a fraction of the maximum respiratory rate. Thus, a value of oxygen satisfaction below unity implied that the dissolved oxygen concentration was below the critical level. An example of the effect of dissolved oxygen on secondary metabolism is provided by Zhou *et al.* (1992) work on cephalosporin C synthesis by *Cephalosporium acremonium*. These workers demonstrated that the critical oxygen concentration for cephalosporin C synthesis during the production phase was 20% saturation. At dissolved oxygen concentrations below 20% cephalosporin C concentration decline and penicillin N increased. Bartholomew *et al.* (1950) represented the transfer of oxygen from air to the cell, during a fermentation, as occuring in a number of steps as transfer of oxygen from air bubble in to solution, then transfer of dissolved oxygen through fermentation media to the microbial cell, finally uptake of dissolved oxygen by the cell.

Many methods for determination of K_La in submerged fermentation have been performed with water and other model fluids, in order to mimic as closely as possible conditions encountered in fermentation systems. These investigations are very useful because conditions are well defined and can be rigorously controlled, and provide fairly good estimates of the oxygen mass transfer that can be used in design calculation. The determination of oxygen absorption from air to fermentation broth should, however, be assessed under actual operating conditions of fermentors since the rate of oxygen absorption into a culture medium can be greatly affected by presence of microorganisms, substrate, substances excreted by microorganisms and antifoam. K_La values in fermentors often differ substantially from values predicted for oxygen absorption in water.

In submerged fermentation, four methods are available to estimate the overall oxygen mass transfer coefficient: Sulphite oxidation method, Static gassing out method, Dynamic gassing out method and Oxygen balance

technique.

In principle, the value of $K_L a$ obtained should be independent of the method employed and $K_L a$ values estimated with the dynamic and steady state methods should be indeed identical. In fact, this ideal situation is rarely met and each method provides a distinct estimation of $K_L a$. This may lead to some design problems in scaling-up fermentors if the scale up method is based on constant oxygen mass transfer coefficient.

In the present investigation the effect of agitation and aeration has been studied on oxygen mass transfer coefficient in 5L fermentor. Dynamic gassing out method was used to estimate the oxygen mass transfer coefficient. The study has been carried out on *Pichia pastoris* in 5L fermentor.

MATERIALS AND METHODS

Fermentation

The fermentor was sterilized by clean steam and prepared for inoculation.Then the media TSB was added through the transfer port by using peristaltic pump. Yeast culture (*Pichia pastoris*) was innoculated in a sterile manner. Proper aeration and agitation was given for growth of organism.

Preparation of preinoculum:

A single colony of yeast from LB-amp agar was transferred to 20ml of pre inoculum (LB) containing - 50µg/ml of ampicillin. The flask was incubated at 250 rpm, 37^0C for seven hours. After two hours of incubation, OD was checked for the samples at every one hour interval.

Preparation of inoculum:

Each 4ml of preinoculum at OD_{600} OF 2.28 was transferred to the three flasks, containing 100ml inoculum medium (MMBL) with 50µg/ml of ampicillin. The flask were incubated at 250 rpm, 37^0C for seventeen hours.

Batch fermentation process:

Prepared three litre MMBL-amp media (50µg of ampicillin/ml of media) as per the composition and transferred to the 5litre fermentor. *In situ* sterilization of fermentor (full vessel sterilization in place) was done.Seeded the fermentor with 10% of inoculum (300ml) at OD_{600} OF 4.22.The process was then started with optimum culture condition of temperature at 37^0C, pH at 7.2 and 100% Dissolved O_2 (DO) was controlled to a minimum of 40% by impeller speed and varying the air inlet by rotometer.The samples were checked for the OD_{600} reading at 1hr interval for continuous six hrs. The wet weight of the samples was estimated. The culture was induced with IPTG at the highest OD of 7.5. The fermentation was continued for about 24hrs.

Spectrophotometry (OD measurement)

Calibrate the instrument with medium as a blank. Each sample was diluted to 1:10 ratio and vortexed. 1.5 ml of each diluted sample was taken in a cuvette and OD was estimated at 600nm,

Dynamic gassing out method (Taguchi and Humphrey 1966):

The fermentor is inoculated with the organism *Pichia pastoris*(300 ml) in 3 litre TSB media. In the presence of respiring organisms, at time t_0, turn OFF air supply to vessel. Monitor and record reduction in DO between time t_0 and t_1. At time t_1, turn the air supply ON again. Monitor and record the rise in DO. Tabulate the readings and plot a graph of t (vs) $\ln((c^*- c)/c^*-c_1)$.

RESULTS AND DISCUSSION

$K_L a$ Estimation (Taguchi and Humphrey 1966)

Estimation of Mass transfer Coefficient ($K_L a$) was done by Dynamic Gassing out method. This experiment was performed on 5L Fermentor.

Condition 1 :	
Fermentor	5 lt
Working volume	3 lt
Temperature	30° C
Aeration	1vvm
Agitator speed	200rpm
Culture	*Pichia pastoris*

In the first experiment the agitation was kept at 200rpm and aeration at 1vvm. It was observed that after stopping the aeration, DO value dropped from 68.3% to 39.4% in 480 seconds. As shown in Fig. 1 the DO value then increased from 39.4% to 65.2% in 700 seconds. From the Fig. 2 which is a plot of dc/dt Vs time, the $K_L a$ value can be estimated using the slope. In this experiment the

Condition 2 :	
Fermentor	5 lt
Working volume	3 lt
Temperature	30° C
Aeration	1vvm
Agitator speed	300rpm
Culture	*Pichia pastoris*

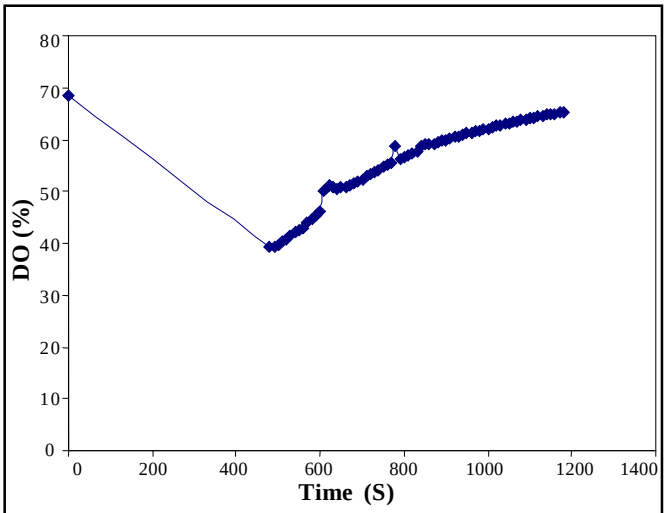

Fig. 1 : Dissolved oxygen profile at 200 rpm and 1vvm

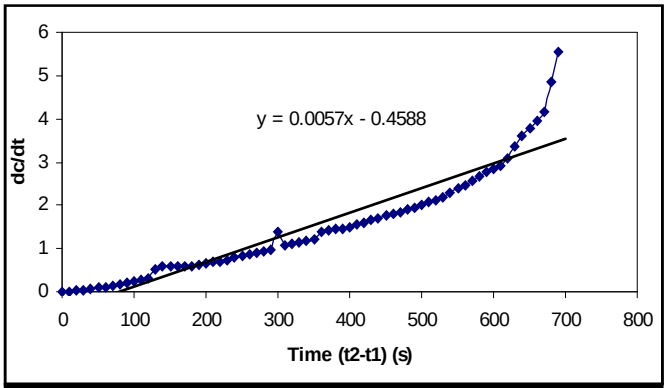

Fig. 2 : $K_L a$ determination at 200rpm and 1vvm

agitation speed was kept at 200rpm and aeration at 1vvm. The $K_L a$ value obtained from the graph was 20.52 per hour. The OUR value can be calculated from the slope of the declining line from Fig. 3. The OUR value in this experiment was 216.72 per hour.

In the second experiment it was observed that after

Condition 3 :	
Fermentor	5 lt
Working volume	3 lt
Temperature	30° C
Aeration	1.5vvm
Agitator speed	300rpm
Culture	*Pichia pastoris*

stopping the aeration, DO value dropped from 89.4% to 40.6% in 563 seconds. As shown in Fig. 4 the DO value then increased from 40.6% to 94.6% in 360 seconds. From the above Fig. 5 which is a plot of dc/dt Vs time, the $K_L a$ value can be estimated using the slope. In this experiment the agitation speed was kept at 300rpm and aeration at 1vvm. The $K_L a$ value obtained from the graph was 169

Table 1: $K_L a$ determination at 200rpm and 1vvm

Time(sec)	DO%	Time (t2-t1) (S)	ln((C*-C1)/(C*-C2))
0	39.2	0	0
10	39.4	10	0.01
20	39.8	20	0.02
30	40.2	30	0.04
40	40.8	40	0.06
50	41.4	50	0.09
60	42	60	0.11
70	42.7	70	0.14
80	43	80	0.16
90	44	90	0.20
100	44.6	100	0.23
110	45.3	110	0.27
120	46.1	120	0.31
130	50	130	0.54
140	51	140	0.60
150	50.7	150	0.58
160	50.6	160	0.58
170	50.7	170	0.58
180	50.9	180	0.60
190	51.2	190	0.62
200	51.6	200	0.65
210	52	210	0.68
220	52.4	220	0.71
230	52.8	230	0.74
240	53.3	240	0.78
250	53.7	250	0.82
260	54.2	260	0.86
270	54.6	270	0.90
280	55	280	0.94
290	55.5	290	0.99
300	58.8	300	1.40
310	56.2	310	1.06
320	56.5	320	1.09
330	56.8	330	1.13
340	57.3	340	1.19
350	57.6	350	1.23
360	58.7	360	1.39
370	59	370	1.43
380	59.1	380	1.45
390	59.2	390	1.47
400	59.4	400	1.50
410	59.7	410	1.55
420	60	420	1.61
430	60.3	430	1.67
440	60.5	440	1.71
450	60.7	450	1.75
460	60.9	460	1.80
470	61.1	470	1.85

Table 1 contd….

Contd…. Table 1

480	61.3	480	1.90
490	61.5	490	1.95
500	61.7	500	2.01
510	62	510	2.09
520	62.1	520	2.13
530	62.3	530	2.19
540	62.6	540	2.30
550	62.8	550	2.38
560	63	560	2.47
570	63.2	570	2.56
580	63.4	580	2.67
590	63.6	590	2.79
600	63.7	600	2.85
610	63.8	610	2.92
620	64	620	3.08
630	64.3	630	3.36
640	64.5	640	3.61
650	64.6	650	3.77
660	64.7	660	3.95
670	64.8	670	4.17
680	65	680	4.87
690	65.1	690	5.56

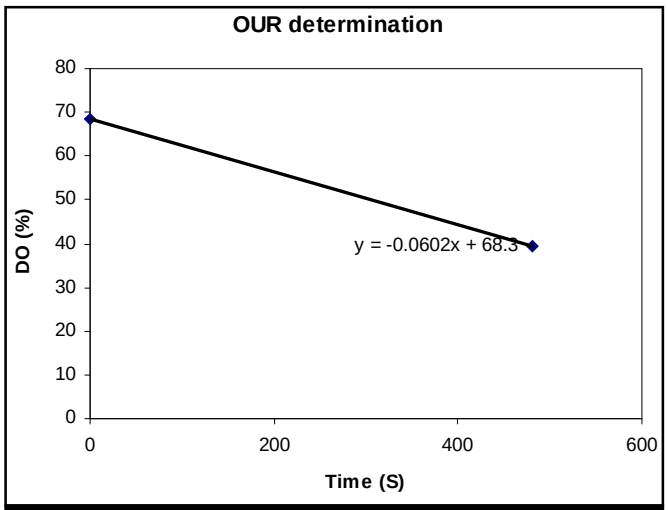

Fig. 3 : OUR determination at 200rpm and 1vvm

per hour. The OUR value can be calculated from the slope of the declining line from Fig. 6. The OUR value in this experiment was 312.12 per hour.

In the third experiment it was observed that after stopping the aeration, DO value dropped from 81.2% to 40% in 570 seconds. As shown in figure 7 the DO value then increased from 40% to 70.7% in 60 seconds.From the above Fig. 8 which is a plot of dc/dt Vs time, the $K_L a$ value can be estimated using the slope. In this experiment the agitation speed was kept at 300rpm and aeration at

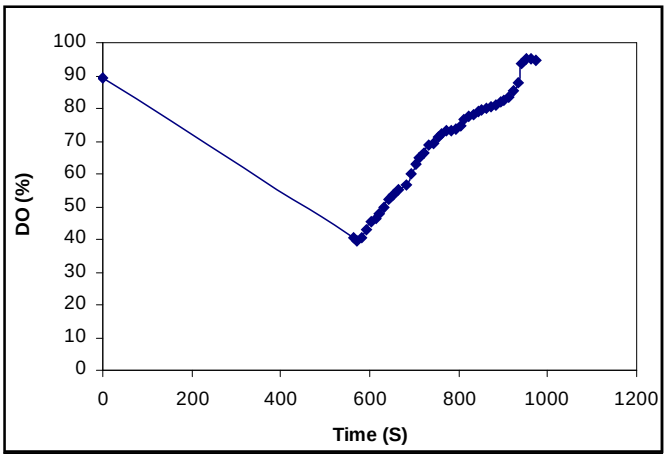

Fig. 4 : Dissolved oxygen profile at 300 rpm and 1 vvm

Table 2 : $K_L a$ determination at 300rpm and 1vvm

Time(sec)	DO%	Time (t2-t1) (S)	ln((C*-C1)/(C*-C2))
10	39.6	0	0
40	45.2	40	0.11
60	47.8	60	0.16
80	52.2	80	0.26
100	55.3	100	0.33
140	62.9	140	0.54
160	66.5	160	0.66
180	69.4	180	0.77
200	72.3	200	0.89
220	73.3	220	0.93
240	74.5	240	0.99
260	77.6	260	1.15
280	78.8	280	1.23
300	79.9	300	1.30
320	81.1	320	1.38
340	82.6	340	1.49
360	85.3	360	1.73

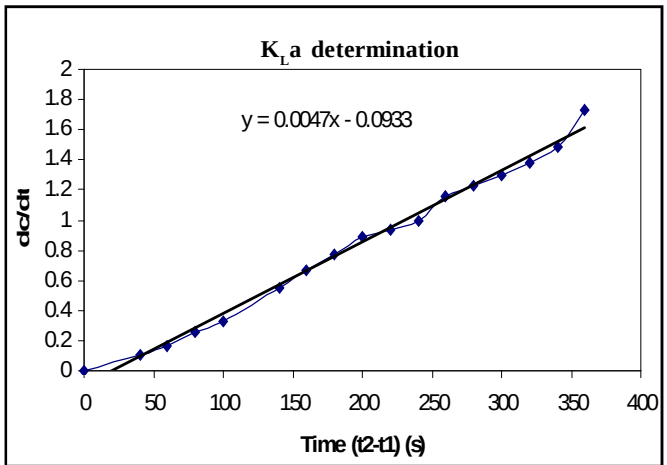

Fig. 5 : $K_L a$ determination at 300 rpm and 1vvm

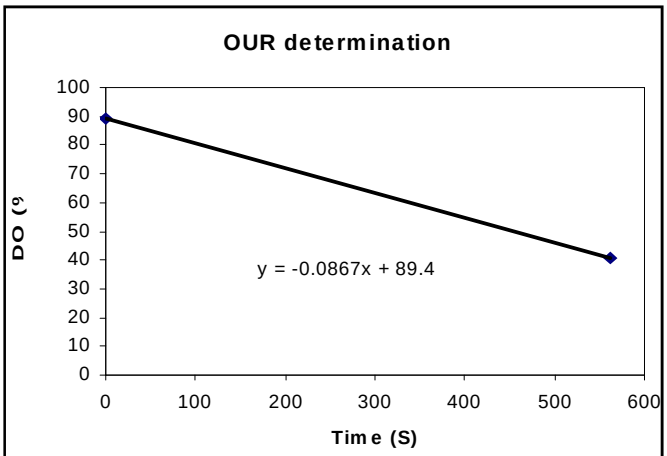

Fig. 6 : OUR determination at 300 rpm and 1 vvm

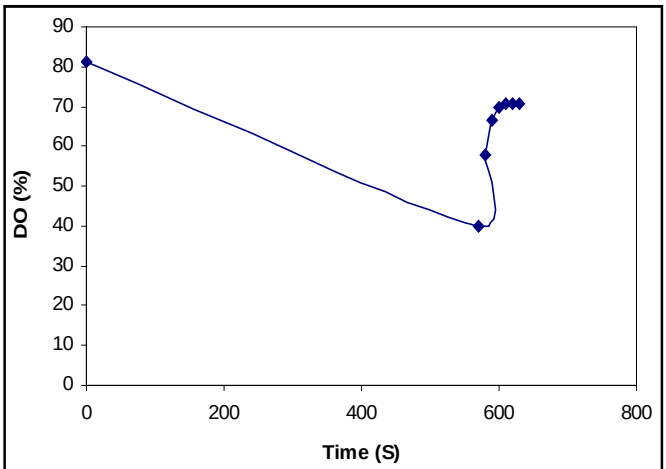

Fig. 7 : Dissolved oxygen profile at 300 rpm and 1.5 vvm

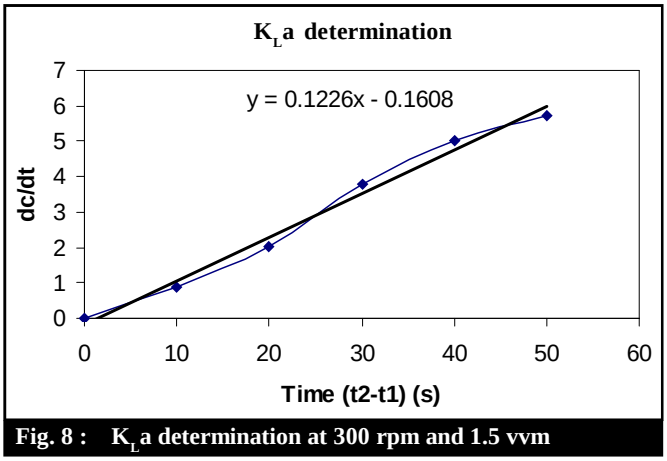

Fig. 8 : K_La determination at 300 rpm and 1.5 vvm

1.5 vvm. The K_La value obtained from the graph was 441.36 per hour. The OUR value can be calculated from the slope of the declining line from Fig. 9. The OUR value in this experiment was 260.28 per hour. The comparision of K_La values at various agitation speed and aeration is depicted in Table 4.

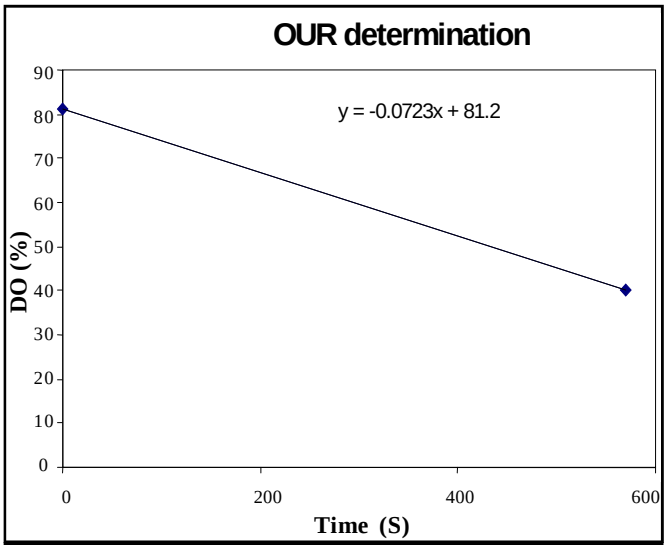

Fig. 9 : OUR determination at 300 rpm and 1.5 vvm

Table 3: K_La determination at 300rpm and 1.5vvm

Time (S)	DO %	Time (t2-t1) (S)	ln((C*-C1)/(C*-C2))
0	40	0	0
10	57.8	10	0.87
20	66.6	20	2.01
30	70	30	3.78
40	70.5	40	5.03
50	70.6	50	5.73

Table 4: Effect of agitation and aeration on K_La values

Air Flow Rate (vvm)		1	1.5
Agitator speed	200	69.12	121
(RPM)	300	169.2	441.36

It can be seen from Fig. 1 that the K_La value increase from 69.2 to 121 as the aeration was increased from 1vvm to 1.5 vvm at 200 rpm. Similar K_La profile

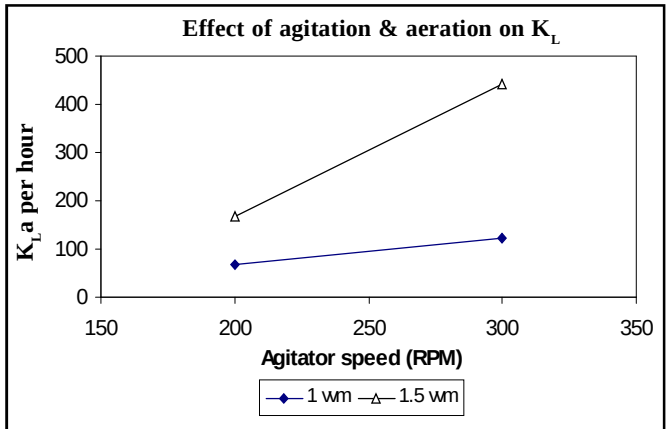

Fig. 10 : Effect of agitation and aeration on K_La values

can also be observed at 300 rpm. Also when the agitation was increased from 200 to 300 rpm an increase in K_La was observed. Hence, it can be concluded that the K_La increases with increase in agitation and aeration. This increase may be attributed to decrease in bubble size due to increase in agitation. Due to increased agitation the bubbles are cut in smaller size and hence the effective surface area of each bubble increases leading to better mass transfer. When the aeration increases the number of bubbles entering the vessel are more and hence, the mass transfer increases. This is possible only until the impellor is not flooded. Once the impeller is flooded the mixing will be governed by the aeration and not agitation hence the K_La results may not indicate the true picture.

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